Title: High Frequency Components of Hemodynamic Shear Stress Profiles Are A Major Determinant of Shear-Mediated Platelet Activation In Therapeutic Blood Recirculating Devices

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SUPPLEMENTARY INFORMATION

The Platelet Activity State (PAS) assay

The extent of activation of HSD-stimulated platelets was quantified via the PAS assay[27]. The PAS assay utilizes as substrate acetylated prothrombin, which upon exposure to damaged activated platelet membranes, as a result of elevated shear exposure, is converted to thrombin that is measured as a surrogate marker of activation[26]. The PAS assay allows characterizing the dynamic response of platelets by measuring the level of activation at increasing number of stimulation cycles[11,12,26-30].

To characterize the dynamics of activation, at each time step (0, 2, 5, and 10 min), 25 µl of GFP drawn from the HSD stimulation chamber were added to a 100-µl tube containing (final concentrations) 5,000 platelets/µl, 200 nM Ac-FII, 5 mM Ca^{2+}, and 100 pM FXa and the tube was incubated at 37°C for 10 min; then, a 10-µl sample was assayed for thrombin generation in a 96-wells microplate reader (Multiskan GO, Thermo Fisher Scientific Inc., Waltham, MA, USA), using 0.3 mM Chromozym-TH (Tosyl-Gly-Pro-Arg-4-nitranilide acetate, Roche Life Science, Milan, Italy) as the thrombin-specific chromogenic peptide substrate. Kinetic absorbance readings were performed at room temperature at 405-nm wavelength for 8 min[29]. The PAS value was calculated as the slope of the linear fitting of the absorbance-time data points over the 8-min kinetic reading. PAS values were normalized against those obtained by sonicating non-stimulated platelets with a microprobe sonicator (HD 2070 Sonoplus, Bandelin Electronic GmbH & Co. KG, Berlin, Germany). The sonication step is meant to yield platelets with maximal prothrombinase activity; thus, normalized PAS values (expressed in percentage, PAS [%]) represent the bulk activity as a fraction of the thrombin generation rate of sonicated platelets (100%)[28]. Sonication conditions (10 W for 10 s) were optimized for bovine platelets starting from the protocol reported for human platelets[28].